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(54) Title: CLEAVAGE OF FUSION PROTEINS USING GRANZYME B PROTEASE

(57) Abstract: A method for the preparation of a polypeptide of interest in authentic form by enzymatic cleavage of fusion proteins using Granzyme B protease (EC 3.4.21.79). There is also provided fusion proteins comprising a polypeptide of interest and a fusion partner, wherein the junction region between the polypeptide of interest and the fusion partner comprises a Granzyme B protease cleavage site adjacent to the polypeptide of interest, and a human Granzyme B protease variant wherein the Cysteine residue no. 228 (chymotrypsinogen numbering) is mutated to Phenylalanine.

## INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/DK2004/000282

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K19/00 C12N9/64

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data, PAJ, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HARRIS J L ET AL: "Definition and redesign of the extended substrate specificity of granzyme B."          THE JOURNAL OF BIOLOGICAL CHEMISTRY. 16 OCT 1998,          vol. 273, no. 42,          16 October 1998 (1998-10-16), pages          27364-27373, XP002296677          ISSN: 0021-9258          cited in the application abstract</p> <p>-----</p> <p style="text-align: center;">-/--</p>	1-39

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

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Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/DK2004/000282

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUN JIURU ET AL: "Importance of the P4' residue in human granzyme B inhibitors and substrates revealed by scanning mutagenesis of the proteinase inhibitor 9 reactive center loop" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 18, 4 May 2001 (2001-05-04), pages 15177-15184, XP002296678 ISSN: 0021-9258 cited in the application abstract -----	1-39
A	WO 03/010204 A (KIM JEONG-MIN ; CHO TAE-HOON (KR); LEE WOO-JONG (KR); PARK HEUNG-BOK () 6 February 2003 (2003-02-06) cited in the application abstract page 3, lines 8-32 page 5, line 29 -----	1-39
A	US 6 010 883 A (THOEGERSEN HANS CHRISTIAN ET AL) 4 January 2000 (2000-01-04) cited in the application abstract column 2, lines 1-40 -----	1-39
A	SUN JIURU ET AL: "Expression and purification of recombinant human granzyme B from <i>Pichia pastoris</i> " BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 261, no. 2, 2 August 1999 (1999-08-02), pages 251-255, XP002296679 ISSN: 0006-291X abstract -----	1-39
A	ROTONDA J ET AL: "THE THREE-DIMENSIONAL STRUCTURE OF HUMAN GRANZYME B COMPARED TO CASPASE-3, KEY MEDIATORS OF CELL DEATH WITH CLEAVAGE SPECIFICITY FOR ASPARTIC ACID IN P1" MOLECULAR CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 8, no. 4, April 2001 (2001-04), pages 357-368, XP001183179 ISSN: 1097-2765 abstract -----	1-39
A	TRAPANI J A: "Granzymes: a family of lymphocyte granule serine proteases." GENOME BIOLOGY 2001, vol. 2, no. 12, 2001, page REVIEWS3014, XP002305250 ISSN: 1465-6914 abstract -----	1-39

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK2004/000282

### Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No
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Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 03010204	A	06-02-2003	EP WO KR	1417237 A1 03010204 A1 2003010536 A		12-05-2004 06-02-2003 05-02-2003
US 6010883	A	04-01-2000	AT AU AU CA DE DE DK EP GB JP JP JP	81529 T 585857 B2 4247485 A 1340381 C 3586750 D1 3586750 T2 217985 A 0161937 A2 2160206 A ,B 1973695 C 7004253 B 61135591 A		15-10-1992 29-06-1989 21-11-1985 09-02-1999 19-11-1992 22-04-1993 17-11-1985 21-11-1985 18-12-1985 27-09-1995 25-01-1995 23-06-1986